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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Flechtner et al.	Confirmation No.:	7221
Serial No.:	10/820,067	Art Unit:	1648
Filed:	April 8, 2004	Examiner:	Blumel, Benjamin P.
For:	HEAT SHOCK PROTEIN-BASED VACCINES AND IMMUNOTHERAPIES	Attorney Docket No:	8449-406-999

DECLARATION OF DR. JESSICA B. FLECHTNER UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Jessica B. Flechtner, do declare that:

1. I am a citizen of the United States of America residing at 30 Old Mill Road, Maynard, Massachusetts.

2. I presently hold the position of Senior Scientist/Group Leader at Genocea Biosciences, Inc. I held the position of Senior Scientist at Antigenics, Inc. from 2003 to 2005. Antigenics, Inc. is the owner of the entire right, title and interest in, to and under the invention described and claimed in the above-identified patent application. I was employed by Mojave Therapeutics, Inc., the original Assignee of the above-identified patent application, from 2001 to 2003, and held the positions there of Scientist and subsequently Scientist II.

3. I received the degrees of B.S. in Animal Science and Ph.D. in Immunology from Cornell University.

4. My academic and technical experience and honors, and a list of my publications are set forth in my *curriculum vitae*, attached hereto as Exhibit 1.

5. I am a co-inventor of the invention described and claimed in the above-identified application, United States Patent Application No. 10/820,067 ("the '067 application"). I have read and understand the '067 application and the Office Action dated September 21, 2006 ("the Office Action") as well as the reference (Wieland et al., United States Patent Application Publication No. US 2004/0071656 A1, "Wieland") cited by the Examiner as a basis for the rejection under 35 U.S.C. § 103(a).

6. I understand that the claims of the '067 application are subject to a rejection wherein the Examiner contends that Wieland would motivate and make obvious the use of the peptide linkers claimed in the '067 application, in developing a hybrid antigen bound to hsc70.

7. I understand that the claims of the '067 application are being amended so as to be directed to a hybrid antigen comprising at least one antigenic domain of an infectious agent or tumor antigen, at least one binding domain that non-covalently binds to a heat shock protein, and at least one peptide linker there between, wherein the peptide linker is Phe Phe Arg Lys ("FFRK peptide linker"); and related compositions and methods.

8. Wieland discloses a hybrid antigen comprising an antigenic domain, a heat shock protein binding domain, and optionally a short peptide linker interposed therebetween (see Wieland at ¶20). In particular, the short peptide linker is a Gly-Ser-Gly ("GSG") peptide linker (see Wieland at ¶158).

9. I will now discuss the evidence indicating the unexpected result achievable with the use of the claimed hybrid antigen of the '067 application. The unexpected result is

that immunization with a hybrid antigen comprising the FFRK peptide linker, the hybrid antigen being alone or noncovalently complexed to a heat shock protein, is more effective at inducing an immune response against the antigenic domain of the hybrid antigen than immunization with a comparable hybrid antigen comprising the GSG peptide linker instead of the FFRK peptide linker, noncovalently complexed to a heat shock protein. In my judgment and opinion, an ordinarily skilled researcher in the field would not have expected that substituting the GSG peptide linker with the FFRK peptide linker would significantly affect the immunogenic activity of the hybrid antigen when used alone or in a noncovalent complex with a heat shock protein.

10. The '067 application presents the data showing the unexpected result described in Paragraph 9 above. Example 4 (pages 60-61) of the '067 application shows the unexpected result that immunization with a hybrid antigen comprising the FFRK peptide linker noncovalently complexed to Hsp70, is more effective at inducing an immune response against the antigenic domain of the hybrid antigen than immunization with a comparable hybrid antigen comprising the GSG peptide linker noncovalently complexed to Hsp70. In the interferon-γ (IFN-γ) ELISPOT assay of Example 4, the immunogenic activity of hybrid antigens containing either the FFRK peptide linker ("the FFRK hybrid antigen") or the GSG peptide linker ("the GSG hybrid antigen") were compared. Both hybrid antigens contained the same heat shock protein binding domain having the amino acid sequence NLLRLTGW and antigenic domain having the amino acid sequence SIINFEKL, a MHC Class I ovalbumin epitope. Mice were immunized subcutaneously at the base of the tail with Hsp70 alone, Hsp70 noncovalently complexed with the SIINFEKL epitope, or Hsp70 noncovalently complexed with the FFRK hybrid antigen or the GSG hybrid antigen. Seven days after immunization, $CD8^+$ T cells were purified from the harvested spleens of the immunized mice

and were assayed in an IFN-γ ELISPOT assay to measure IFN-γ secretion by $CD8^+$ T cells upon exposure to the SIINFEKL epitope (see the '067 application at pages 60-61). IFN-γ secretion by $CD8^+$ T cells upon recognition of the target antigen is an indication of $CD8^+$ T cell activation and specific $CD8^+$ T cell response against the target antigen. Results of the IFN-γ ELISPOT assay are reported in the table in Example 4 as the average number of spots per 400,000 $CD8^+$ T cells from at least 4 experiments (the '067 application at pages 60-61). Controls included medium alone, unpulsed T cells, T cells pulsed with a non-immunized peptide derived from VSV (negative control), or T cells pulsed with Concanavalin A (positive control) (see the '067 application at pages 60-61). As shown in the table in Example 4, approximately three times more spots were detected in samples with $CD8^+$ T cells from mice immunized with the FFRK hybrid antigen noncovalently complexed to Hsp70 than in samples with $CD8^+$ T cells from mice immunized with the GSG hybrid antigen noncovalently complexed to Hsp70, when the $CD8^+$ T cells were pulsed with the SIINFEKL epitope. The ^{51}Cr-release assay result in Example 4 is consistent with the IFN-γ ELISPOT result. Target cells were intracellularly labeled with the ^{51}Cr radioisotope, pulsed with the SIINFEKL epitope, and cultured with $CD8^+$ T cells purified from the same immunized mice used in the ELISPOT assay described above. Target cells that are recognized and killed by $CD8^+$ T cells release ^{51}Cr into the media, and the amount of ^{51}Cr released into the media was used to calculate the percentage of target cell killing at a ratio of 200 effector $CD8^+$ T cells per one target cell. As shown in the right column of the table in Example 4, $CD8^+$ T cells isolated from mice immunized with the FFRK hybrid antigen noncovalently complexed to Hsp70 achieved a higher percentage of target cell killing than $CD8^+$ T cells isolated from mice immunized with the GSG hybrid antigen noncovalently complexed to Hsp70.

11. Examples 5 (pages 61-62) and 6 (pages 62-63) of the '067 application show the unexpected result that the FFRK hybrid antigen, alone or noncovalently complexed to Hsp70, is better than the GSG hybrid antigen noncovalently complexed to Hsp70 in eliciting a specific $CD8^+$ T cell response against the SIINFEKL epitope of the antigenic domain. Examples 5 and 6 show the results of IFN-γ ELISPOT assays performed similarly as described in Paragraph 10 above. As shown in the tables in Examples 5 and 6, $CD8^+$ T cells from mice immunized with the FFRK hybrid antigen, alone or noncovalently complexed to Hsp70, produced a higher response that is specific to the SIINFEKL epitope of the antigenic domain than $CD8^+$ T cells from mice immunized with the GSG hybrid antigen noncovalently complexed to Hsp70. In Example 5, immunization with the FFRK hybrid antigen alone or noncovalently complexed to Hsp70 resulted in approximately a 12-fold and 64-fold increase, respectively, in $CD8^+$ T cell response to the SIINFEKL epitope relative to immunization with the GSG hybrid antigen noncovalently complexed to Hsp70 (the '067 application at page 62). In Example 6, immunization with the FFRK hybrid antigen alone or noncovalently complexed to Hsp70 resulted in approximately a 1.6-fold and 5-fold increase, respectively, in $CD8^+$ T cell response to the SIINFEKL epitope relative to immunization with the GSG hybrid antigen noncovalently complexed to Hsp70 (the '067 application at page 63).

12. The evidence described in Paragraphs 10 and 11, in my opinion and judgment, clearly shows that immunization with a hybrid antigen comprising the improved FFRK peptide linker of the '067 application, alone or noncovalently complexed to a heat shock protein, is unexpectedly more effective at inducing an immune response to the antigenic domain epitope of the hybrid antigen relative to immunization with a hybrid antigen comprising the conventional GSG peptide linker and noncovalently complexed to a heat shock protein.

13. I declare further that all statements made in this Declaration of my knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 15 March 07

Jessica B. Flechtner, Ph.D.

Attachment:

Exhibit 1: *Curriculum Vitae* of Jessica B. Flechtner, Ph.D.